COMMUNICATION

Determination of Enantiomeric Excess in Amine Derivatives Via Molecular Self-Assemblies

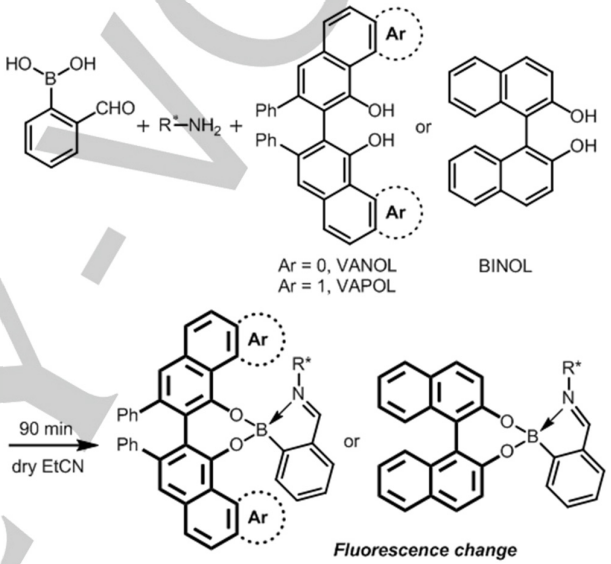
Elena G. Shcherbakova,[a] Tsuyoshi Minami,[a] Valentina Brega,[a] Tony D. James,[b] Pavel Anzenbacher, Jr.*[a]

Abstract: We report the first fluorescence-based assay for rapid *e.e.* determination of amines, amino alcohols, and amino acid esters. The method uses the self-assembly of 2-formylphenylboronic acid with a chiral diol and a chiral amine or derivatives (of unknown chirality) to produce two diastereomeric iminoboronates that differ in their fluorescence intensity and polarization. The approach allows for the accurate determination of the *e.e.* of chiral amines with errors of just 1-2%. We believe that this application of orthogonal dynamic covalent self-assembly in *e.e.* determination will lead to the development of high throughput protocols for determination of chirality.

Chiral amines play pivotal roles in biology,[1] pharmaceutical chemistry[2] and are used as chiral auxiliaries.[3] Given their importance, determination of enantiomeric excess (*e.e.*) have become central to organic and medicinal chemistry and necessitates the development of methods for fast and reliable determination of *e.e.*.[4]

One of the most common methods for the determination of enantiomeric purity is the measurement of optical rotation.[5] In addition, NMR spectroscopy,[6] GC and HPLC are also useful techniques.[7] As an alternative, circular dichroism (CD) has been used and recently brought to high-throughput level.[8] Alas, these methods require high concentrations of analytes, strict conditions, and – with few exceptions – are too slow for high throughput-screening (HTS).[9]

For the above reasons we have decided to develop a sensitive and HTS-amenable assay for chiral amines and their derivatives. We take advantage of a ternary system comprising an *α*-chiral primary amine, 2-formylphenylboronic acid (FPBA), and enantiomerically pure 1,1'-bi-2-naphthol (BINOL), which assemble rapidly, leading to characteristic ^{1}H-NMR spectroscopic shifts for each enantiomer of the amine (Scheme 1).[10] This system has also been employed for *e.e.* determination using electrochemical[11] or CD methods.[12] Our assumption is that the twist angle of the fluorescent BINOL ligand, is affected by the chirality of the analyte during the assembly resulting in detectable fluorescence changes (*i.e.* intensity changes and spectral shifts). We have selected several aromatic diol derivatives: BINOL, 3,3'-Diphenyl-2,2'-bi-1-naphthol (VANOL), and 2,2'-diphenyl-(4-biphenanthrol) (VAPOL), as the reporter moiety for the analysis of chiral amines, amino alcohols, and amino acids. VANOL and VAPOL are more sterically encumbering ligands, and are thus more likely to increase the enantioselectivity in the formation of the resulting iminoboronate esters. Here, the fluorescence from the diastereomeric product mixture (*S*-ligand, chiral amine with varying *e.e.*, achiral 2-formylphenylboronic acid, FPBA, Scheme 1) is read rapidly using a microplate reader.



Scheme 1. Three-component self-assembly reaction between 2-formylphenylboronic acid, a primary amine, and aromatic diol derivatives.

The reaction in Scheme 1 was studied using mass spectrometry (see Supporting Information), fluorescence spectroscopy and single crystal X-ray diffraction analysis. Figure 1 shows the X-ray structures of two enantiomeric iminoboronate esters (*S*,*S* and *R*,*R*) of FPBA, α-methyl benzylamine (MBA), and VAPOL in 1:1:1 stoichiometry. Figure 1 also shows the pseudotetrahedral geometry at the boron atom. Such a geometry provides rigidity, which is a factor both in governing the enantioselectivity of the system and in communicating any binding events to the fluorophore.

Next, a quantitative determination of chiral enrichment in amines, amino alcohols, and amino acids (in the form of methyl ester) was obtained by fluorescence titrations. An example is shown in Figure 2. Here, the fluorescence of *S*-VANOL is quenched upon formation of the iminoboronate ester. Figure 2 (Top) shows the attenuation of the fluorescence upon addition of *R*-methylbenzylamine. Figure 2 (Bottom) shows two isotherms corresponding to the fluorescence response to *R*- and *S*-

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methylbenzylamine. The quenching is likely due to photoinduced electron transfer from the nitrogen of the complexes.[13] In the presence of amino alcohols, a fluorescence red-shift due to the formation of oxazolidine boronate ester is observed.[14] Notably, the changes in the fluorescence signal between diastereomers are different, suggesting that the determination of enantiomeric excess can be monitored using changes in fluorescence intensity and polarization.

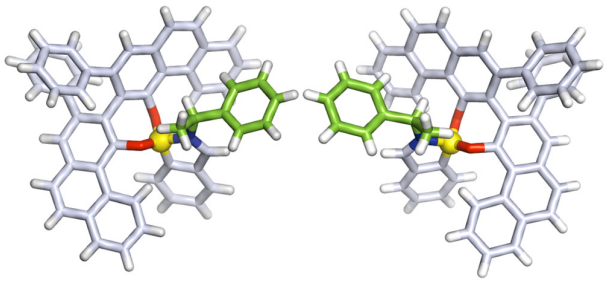

Figure 1. X-ray crystal structures of (**left**) (*S*,*S*)- and (**right**) (*R*,*R*)-iminoboronate esters self-assembled from FPBA, MBA, and VAPOL. The carbon atoms are shown in gray (or green for MBA), the boron in yellow, the nitrogen in blue, the oxygen in red, and the hydrogen atoms in white.

Importantly, the two diastereomeric complexes (*S*)-VANOL+(*S*)-methylbenzylamine (+FPBA) vs. (*S*)-VANOL+(*R*)-methylbenzylamine (+FPBA) show different fluorescence intensities, as well as different association isotherms and apparent association constants (K_a). On the other hand, a control experiment utilizing two enantiomeric mixtures (*S*)-BINOL+(*S*)-methylbenzylamine (+FPBA) vs. (*R*)-BINOL+(*R*)-methylbenzylamine (+FPBA) showed identical isotherms.

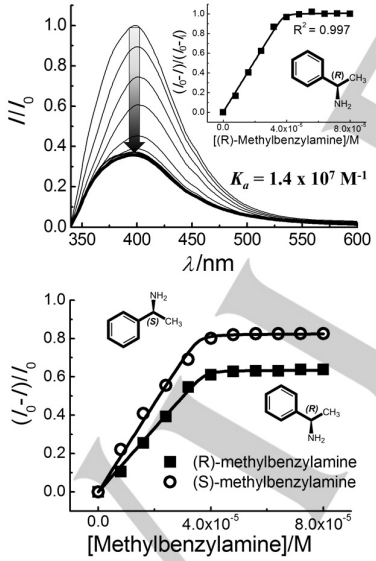

Figure 2. Top: Fluorescence spectra of (*S*)-VANOL (40 μM) with FPBA (40 μM) upon addition of (*R*)-methylbenzylamine in dry EtCN. λ_{ex} = 335 nm. [(*R*)-methylbenzylamine] = 0 - 80 μM. **Bottom:** Binding isotherm for (*S*)- and (*R*)-methylbenzylamine show distinctly different behavior of both enantiomers.

Table 1 shows the apparent association constants (K_a, M^{-1}) for selected amines, amino alcohols, and amino acid esters. Importantly, the method is not dramatically sensitive to potential impurities such as water (up to 5% v/v after which two phases separate), ethanol (tested up to 50% v/v), ethylene glycol, glucose and citrate (all up to 4.0 mM in water, i.e. 1 equivalent).

The values in Table 1 suggest that the K_a are different between enantiomeric amines, thus enabling the reliable detection of *e.e.*. The general order of association constants observed was amines ~ amino alcohols > amino acid esters. These differences then contribute to the discrimination of analytes in an array-based assay.

Table 1. The apparent association constants (K_a, M^{-1})[a] obtained from fluorescence titration in dry EtCN.

Guest	(*S*)-BINOL K_a, M^{-1}	(*S*)-VANOL K_a, M^{-1}	(*S*)-VAPOL K_a, M^{-1}
(*S*)-methylbenzylamine (**MBA**)	7.0 x 10^6	2.3 x 10^7	3.1 x 10^6
(*R*)-methylbenzylamine	2.4 x 10^6	1.4 x 10^7	4.5 x 10^6
(*S*)-methylphenethylamine (**MPA**)	9.9 x 10^5	> 10^8	1.9 x 10^6
(*R*)-methylphenethylamine	5.6 x 10^5	> 10^8	1.3 x 10^6
(*S*)-2-aminobutane (**AMB**)	4.2 x 10^5	1.5 x 10^7	7.9 x 10^6
(*R*)-2-aminobutane	1.2 x 10^6	1.2 x 10^6	4.0 x 10^6
(*S*)-1-(2-naphthyl) ethylamine (**NEA**)	2.4 x 10^6	8.0 x 10^6	1.6 x 10^6
(*R*)-1-(2-naphthyl) ethylamine	2.2 x 10^6	3.6 x 10^6	2.1 x 10^6
(*S*)-phenylalanine (**Phe**)	4.1 x 10^3	3.3 x 10^4	2.0 x 10^4
(*R*)-phenylalanine	5.9 x 10^3	1.3 x 10^5	3.2 x 10^4
(*S*)-valine (**Val**)	5.4 x 10^3	7.4 x 10^4	2.6 x 10^4
(*R*)-valine	7.2 x 10^3	1.1 x 10^4	3.1 x 10^4
(*S*)-2-amino-3-phenyl-1-propanol (**APP**)	9.7 x 10^5	5.8 x 10^6	1.7 x 10^6
(*R*)-2-amino-3-phenyl-1-propanol	1.5 x 10^7	3.0 x 10^6	6.7 x 10^6

[a] The K_a values were calculated based on the change in fluorescence intensity upon addition of each guest. The errors in the curve fitting were < 20%.

The fluorescence data were recorded using a conventional plate reader and 384-well microplates (see the SI). Pattern recognition protocols[15] were then used to reveal the guest-specific trends in the overall response.

First, we performed a qualitative assay, run at a constant concentration of 40 μM and 1:1:1 stoichiometry of the amine, chiral auxiliary and FPBA. The qualitative assay focused on the difference in fluorescence between the two enantiomeric amines.

A large difference in the spectroscopic behavior of the enantiomers is manifested as a large distance between the corresponding clusters in the linear discriminant analysis (LDA)[16] plot (Figure 3). LDA is a frequently used supervised pattern recognition method for reduction of dimensionality and classification of the multivariate data. LDA models the similarity by maximizing the distance between the classes and minimizing the distance between the trials within the clusters. Cross-validation procedure is performed to ascertain the level of correct classification of the observations within the clusters. From the score plot one can see that the responses could discriminate 16 analytes and a control with 100% correct classification of all 340 data-points (corresponding to 16 analytes and a control). The assay recognized the amino-derivatives and sorted them into three groups: amines, amino alcohols, and amino acids. Importantly, the obtained results reveal that the assay can discriminate different enantiomers of amines, amino alcohols, and amino acids and that the (*S*)-isomers (circles) are well separated from the (*R*)-isomers (squares).

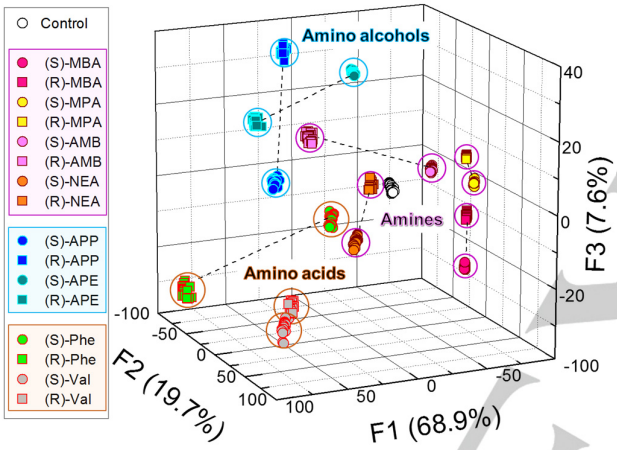

Figure 3. Results of the qualitative linear discriminant analysis (LDA) of amine, amino alcohol, and amino acid enantiomers in EtCN using a fluorescence assay show large distances between the clusters of enantiomer pairs suggesting a high chance for determination of *e.e.*. (MBA: methylbenzylamine, MPA: methylphenethylamine, AMB: 2-aminobutane, NEA: 1-(2-naphthyl) ethylamine, APP: 2-amino-3-phenyl-1-propanol, APE: 2-amino-1-phenylethanol.

The following semi quantitative assay (Figure 4) shows results of analyses of varying *e.e.* for four different amino derivatives: methylbenzylamine, 1-(2-naphthyl)ethylamine, 2-amino-3-phenyl-1-propanol and phenylalanine. All four compounds were measured in 10 steps of varying enantiopurity, from 0 to 100 % *e.e.*. Once again, a 100% correct classification is observed. The linear trends in the individual series of the responses showed smooth progression from 0 to 100% *e.e.*, suggesting a high chance for successful linear regression analysis of *e.e.*.

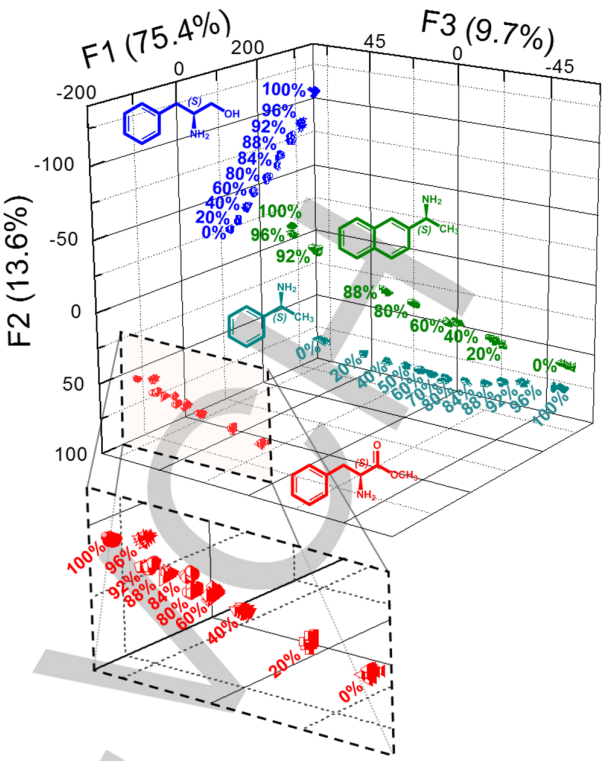

Figure 4. Results of LDA in EtCN for the semi-quantitative assay of (*S*)-methylbenzylamine (**Cyan**), (*S*)-1-(2-naphthyl)ethylamine (**Green**), (*S*)-2-amino-3-pheyl-1-propanol (**Blue**), (*S*)-phenylalanine (**Red**).

For the quantitative % e.e. determination analysis of the guests, we used a support vector machine (SVM) regression method, which is more suitable for modeling complex responses and non-linear behavior of the data.[17] Briefly, SVM is a supervised classification method that seeks to separate classes by mapping the input into an n-dimensional vector space using kernel functions. The SVM regression method constructs calibration models serving to predict the e.e. values of unknown samples.

The SVM regression allowed for prediction of multiple points of enantiomeric excess. We used 10 data-points corresponding to various *e.e.* values to model the behavior of the data and two different % *e.e.* values to validate the model. The developed model was used to quantify two unknown samples (squares in Figure 5). The quantitative assay yielded a very accurate % *e.e.* regression analysis of the amino-derivatives as shown by the root-mean-square errors (RMSEs). The RMSE of the prediction (RMSEP) revealed that the assay can predict the % *e.e.* of each analyte with maximum error of 1-2 % (Figure 5).

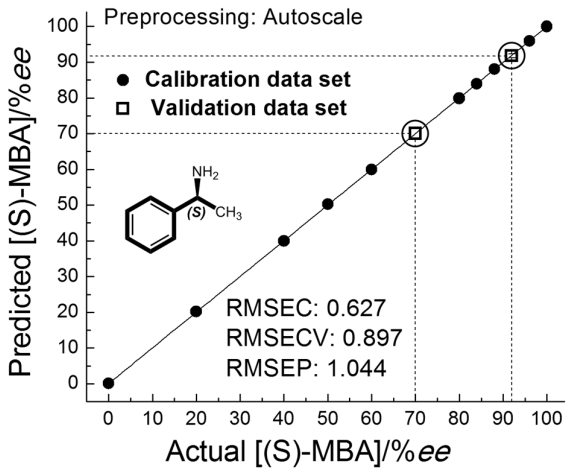

Figure 5. Results of the SVM regression for *e.e.* determination analysis of methylbenzylamine (**MBA**). The plot of actual vs predicted *e.e.* shows high accuracy of prediction for multiple *e.e.*. The values of the root-mean-square errors (RMSEs) of calibration (C), cross-validation (CV), and prediction (P) (shown as squares □) attest to the high quality of the model and prediction.

In summary, we have reported the first fluorescence-based assay for enantiomeric excess determination in amines, amino alcohols, and amino acid esters. This method uses molecular self-assemblies based on boronate esterification. Fluorescence titrations showed highly variable substrate-dependent changes in fluorescence for various percentages of enantiomeric excess. The assay itself requires only a simple fluorescence plate reader and may be performed in high-throughput fashion. Analysis of % *e.e.* with low errors was successfully achieved using support vector machines (SVM). We believe that this application of orthogonal dynamic covalent self-assembly in *e.e.* determination will lead to the development of high throughput protocols for determination of enantiomeric excess.

Experimental Section

1.1 Equivalent of enantiopure aromatic diol derivative (BINOL, VANOL, or VAPOL) and 1 equiv. of 2-formylphenylboronic acid are dissolved in EtCN to form 4 mM stock solution. Subsequently, 1 equiv. of amine of known or unknown e.e. is added and reaction mixture incubated for 90 min to ensure a complete formation of diasteriomeric iminoboronate esters. Each reaction mixture is then diluted by 100 times with pure propionitrile to a final concentration of 40 μM.

The diluted reaction mixtures are dispensed into the microplates and read with a microplate reader with the following sets of filters: Fluorescence intensity (FI): 300 nm excitation / 380 nm emission (1st channel) and 320 nm excitation / 370 nm emission (2nd channel), and fluorescence polarization (FP) at 330 nm excitation and 420 nm dual emission.

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Keywords: Supramolecular Chemistry • Chirality • Self-assembly • Fluorescence • Sensors

[1] *Chirality in Natural and Applied Science* (Eds.: W. J. Lough, I. W. Wainer), Blackwell, Oxford, UK, **2002**.

[2] a) *Chirality in Drug Research* (Eds.: E. Francotte, W. Lindner), Wiley-VCH, Weinheim, **2006**; b) E. L. Izake, *J. Pharm. Sci.* **2007**, *96*, 1659–1676.

[3] J. Seyden-Penne, *Chiral Auxililiaries and Ligands in Asymmetric Synthesis*, Wiley & Sons, New York, **1995**.

[4] a) L. Pu, *Acc. Chem. Res.* **2012**, *45*, 150-163; b) L. Pu, *Chem. Rev.* **2004**, *104*, 1687-1716; c) X. Zhang, J. Yin, J. Yoon, *Chem. Rev.* **2014**, *114*, 4918-4959; d) M. Tsukamoto, H. B. Kagan, *Adv. Synth. Catal.* **2002**, *344*, 453-463; e) G. W. Gokel, W. M. Leevy, M. E. Weber, *Chem. Rev.* **2004**, *104*, 2723-2750; f) K. Ariga, G. J. Richards, S. Ishihara, H. Izawa, J. P. Hill, *Sensors* **2010**, *10*, 6796-6820; g) H. Tsukube, S. Shinoda, H. Tamiaki, *Coord. Chem. Rev.* **2002**, 226, 227-234; h) L. Mutihac, J. H. Lee, J. S. Kim, J. Vicens, *Chem. Soc. Rev.* **2011**, *40*, 2777-2796; i) Y. Kubo, S. Maeda, S. Tokita, M. Kubo, *Nature* **1996**, *382*, 522-524.

[5] P. L. Polavarapu, *Chirality* **2002**, *14*, 768-781.

[6] a) T. J. Wenzel, *Discrimination of Chiral Compounds Using NMR Spectroscopy*, John Wiley & Sons, Hoboken, NJ, **2007**; b) T. J. Wenzel, J. D. Wilcox, *Chirality* **2003**, *15*, 256-270; c) T. Ema, *J. Incl. Phenom. Macrocycl. Chem.* **2012**, *74*, 41-55; d) D. Parker, *Chem. Rev.* **1991**, *91*, 1441-1457.

[7] a) G. Guebitz, M. G. Schmid, *Mol. Biotechnol.* **2006**, *32*, 159-179; b) W. H. Pirkle, T. C. Pochapsky, *Chem. Rev.* **1989**, *89*, 347-362; c) F. Gasparrini, D. Misiti, C. Villani, *J. Chromatogr. A* **2001**, *906*, 35-50; d) Y. Okamoto, E. Yashima, *Angew. Chem.* **1998**, *110*, 1072-1095; *Angew. Chem. Int. Ed.* **1998**, *37*, 1020-1043.

[8] a) G. A. Hembury, V. V. Borovkov, Y. Inoue, *Chem. Rev.* **2008**, *108*, 1-73; b) E. Yashima, K. Maeda, T. Nishimura, *Chem. –Eur. J.* **2004**, *10*, 42-51; c) D. Leung, S. O. Kang, E. V. Anslyn, *Chem. Soc. Rev.* **2012**, *41*, 448-479; d) C. Wolf, K. W. Bentley, *Chem. Soc. Rev.* **2013**, *42*, 5408-5424; e) J. W. Canary, S. Mortezaei, J. Liang, *Coord. Chem. Rev.* **2010**, *254*, 2249-2266.

[9] a) H. H. Jo, C.-Y. Lin, E. V. Anslyn, *Acc. Chem. Res.* **2014**, *47*, 2212-2221; b) J. M. Dragna, G. Pescitelli, L. Tran, V. M. Lynch, E. V. Anslyn, L. Di Bari *J. Am. Chem. Soc.* **2012**, *134*, 4398-4407; c) S. Nieto, J. M. Dragna, E. V. Anslyn, *Chem. –Eur. J.* **2010**, *16*, 227-232.

[10] a) Y. Pérez-Fuertes, A. M. Kelly, J. S. Fossey, M. E. Powell, S. D. Bull, T. D. James, *Nat. Protoc.* **2008**, *3*, 210-214; b) Y. Pérez-Fuertes, A. M. Kelly, A. L. Johnson, S. Arimori, S. D. Bull, T. D. James, *Org. Lett.* **2006**, *8*, 609-612; c) A. M. Kelly, Y. Pérez-Fuertes, S. Arimori, S. D. Bull, T. D. James, *Org. Lett.* **2006**, *8*, 1971-1974; d) D. A. Tickell, M. F. Mahon, S. D. Bull, T. D. James, *Org. Lett.* **2013**, *15*, 860-863; e) A. Wilson, G. Gasparini, S. Matile, *Chem. Soc. Rev.* **2014**, *43*, 1948-1962.

[11] G. Mirri, S. D. Bull, P. N. Horton, T. D. James, L. Male, J. H. R. Tucker, *J. Am. Chem. Soc.* **2010**, *132*, 8903-8905.

[12] a) P. Metola, E. V. Anslyn, T. D. James, S. D. Bull, *Chem. Sci.* **2012**, *3*, 156-161; b) K. W. Bentley, Y. G. Nam, J. M. Murphy, C. Wolf. *J. Am. Chem. Soc.* **2013**, *135*, 18052-18055.

[13] A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515-1566.

[14] E. Galbraith, A. M. Kelly, J. S. Fossey, G. Kociok-Köhn, M. G. Davidson, S. D. Bull, T. D. James, *New J. Chem.* **2009**, *33*, 181-185.

[15] a) J. J. Lavigne, E. V. Anslyn, *Angew. Chem.* **2001**, *113*, 3212-3225; *Angew. Chem. Int. Ed.* **2001**, *40*, 3118-3130; b) E. V. Anslyn, *J. Org.*

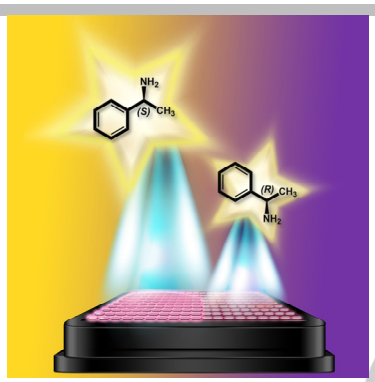
Chem. **2007**, *72*, 687-699; c) K. J. Albert, N. S. Lewis, C. L. Schauer, G. A. Sotzing, S. E. Stitzel, T. P. Vaid, D. R. Walt, *Chem. Rev.* **2000**, *100*, 2595-2626; d) R. Paolesse, D. Monti, F. Dini, C. Di Natale, *Top. Curr. Chem.* **2011**, *300*, 139-174; e) N. A. Rakow, A. Sen, M. C. Janzen, J. B. Ponder, K. S. Suslick, *Angew. Chem.* **2005**, *117*, 4604-4608; f) T. Minami, N. A. Esipenko, B. Zhang, L. Isaacs, P. Anzenbacher, Jr., *Chem. Commun.* **2014**, *50*, 61-63.

[16] a) R. G. Brereton, *Applied Chemometrics for Scientists*, Wiley, Chichester, **2007**; b) P. Anzenbacher, Jr., P. Lubal, P. Buček, M. A. Palacios, M. E. Kozelkova, *Chem. Soc. Rev.* **2010**, *39*, 3954-3979.

[17] a) L. H. Hamel, Knowledge Discovery with Support Vector Machines, Wiley, Hoboken, NJ, **2009**; b) Christianini, N.; Shawe-Taylor, J.; *An Introduction to Support Vector Machines*; Cambridge University Press: Cambridge, U.K., 2000. c) T. Minami, N. A. Esipenko, B. Zhang, M. E. Kozelkova, L. Isaacs, R. Nishiyabu, Y. Kubo, P. Anzenbacher, Jr. *J. Am. Chem. Soc.* **2012**, *134*, 20021-20024.

COMMUNICATION

The dynamic covalent self-assembly of chiral amines with chiral fluorescent diols and 2-formylphenylboronic acid produces fluorescent diastereomeric iminoboronates. The fluorescence-based assay for the rapid enantiomeric excess determination of amine derivatives utilizing this principle requires only simple laboratory instrumentation and is amenable to high-throughput screening.



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